Spaceflight and Bone Turnover:

Correlation with a New Rat Model of Weightlessness

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The near-weightless environment of orbital flight has produced certain biomedical effects in humans including abnormalities in mineral metabolism. Alterations in calcium homeostasis were suggested by early manned spaceflights (Biryukov and Krasnykh 1970, Lutwak et al. 1969, Mack and LaChance 1967, Rambaut et al. 1975), but the most definitive data to date became available following the long-duration flights of the Skylab series (Leach and Rambaut 1977, Vogel et al. 1977, Whedon et al. 1977). The Skylab mineral studies indicated that during flight, urinary calcium increased immediately and stabilized within 30 days at a level about twice the preflight value (Rambaut et al. 1979 and Fig. 1). Fecal calcium decreased initially, then continued to rise throughout the flights with no indication of abating. Calcium balance returned to normal in the postflight period.

These data were compatible with bone mineral loss. The relative densities of the heel bone and the distal end of the radius and ulna were measured before and following flight using photon absorptiometry. Only after 84 days of near weightlessness and only in the heel bone was a significant decrease in bone density observed (Vogel et al. 1977 and Fig. 2). However, no significant loss of urinary hydroxyproline or hydroxylysine glycosides was found throughout the 84-day Skylab flight, indicating no enhancement of bone breakdown (Claus-Walker et al. 1977).

In missions of longer duration, unabated calcium loss could cause not only a decrement in skeletal strength but also perturbations in many physiological systems dependent on calcium for normal function. If complications commonly associated with disuse osteoporosis, such as hypercalcemia, renal stones, and ectopic calcification, occurred during flight, the success of a mission could be severely hampered. Thus, the extent and duration of the calcium loss during flight should be defined prior to extended manned missions.

When the Soviets offered to examine proposals from the USA for possible inclusion among the experiments on the Cosmos 782 biological satellite, investigation of the potential mechanisms involved in changes in bone turnover seemed a logical extension of the Skylab studies. A decreased trabecular mass had been noted in the metaphyses of rats after 22 days onboard the Cosmos 605 spacecraft (Yagodovsky et al. 1976). Bone mass could be lost during spaceflight by decreasing the amount of mineral that goes into bone (bone formation) and/or increasing the amount of mineral that comes out of bone (bone resorption). The site of the defect should be identified so that meaningful countermeasures could be proposed.

To define further the effects of spaceflight on bone, a proposal to study parameters of periosteal bone formation and endosteal bone resorption in the rat tibial diaphysis was submitted and subsequently accepted. In this experiment, young growing rats about 63 days of age at the beginning of flight were injected with a fluorescent bone marker (demeclocycline, a tetracycline derivative) be-

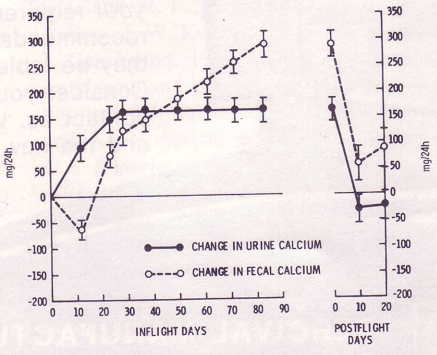

Fig. 1. Change in urine and fecal calcium as a function of Skylab flight duration. Data are expressed as mean ± standard error. (Figure courtesy of Paul C. Rambaut, NASA Johnson Space Center.)

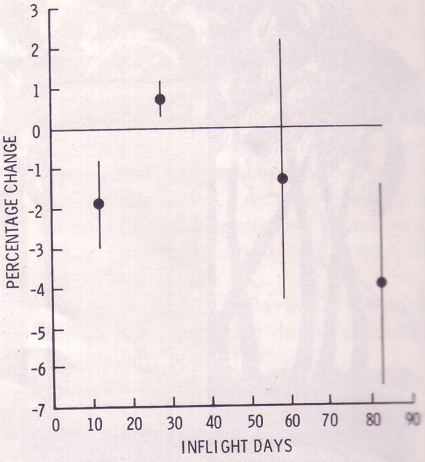

Fig. 2. Postflight bone mineral changes in spaceflight crews. Data are expressed as mean ± standard error. The results of gamma-ray absorptiometric measurements of bone mineral mass are shown. Measurements made before and after the flights of Apollo 14, 15, and 16 comprise the first data point of mineral mass loss after about 13 days in space. The remaining three time periods are measurements made on the Skylab missions. (Figure courtesy of Paul C. Rambaut, NASA Johnson Space Center.)

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fore flight so that bone growth during flight could be measured. A second injection, after flight, was given to quantify bone growth in the month following flight.

COSMOS BONE RESULTS

The most striking effects were those on bone formation (Morey and Baylink 1978). During flight, rats formed significantly less periosteal bone than did ground control animals. An arrest line found around the periosteum of flight animals suggested that cessation of bone growth occurred during spaceflight. Bone formation in these rats probably stopped sometime after the 11th day of near weightlessness since the total bone mass formed during the 19.5-day flight was laid down by the ground control rats in approximately 11 days. Bone formation was apparently reinitiated within 3 days of reentry, and by 26 days after flight the flight rats showed a significant increase in bone formation rate as compared with vivarium controls. No significant changes in bone resorption were observed.

A similar experiment was flown aboard the next Soviet Cosmos biological satellite, 936, which contained both stationary rats and rats on a short-radius centrifuge. Data from this experiment were virtually identical with those from Cosmos 782 (Table 1). Moreover, in Cosmos 936, (a) centrifugation at 1-G during flight did not appear to correct the defect in tibia diaphyseal bone formation although it did accelerate recovery of bone mass after return to earth, and (b) no rebound in bone formation occurred in the postflight period, although bone formation did return to normal if the flight rats were compared with the flight controls (which were similarly confined throughout the flight period) rather than with the vivarium controls.

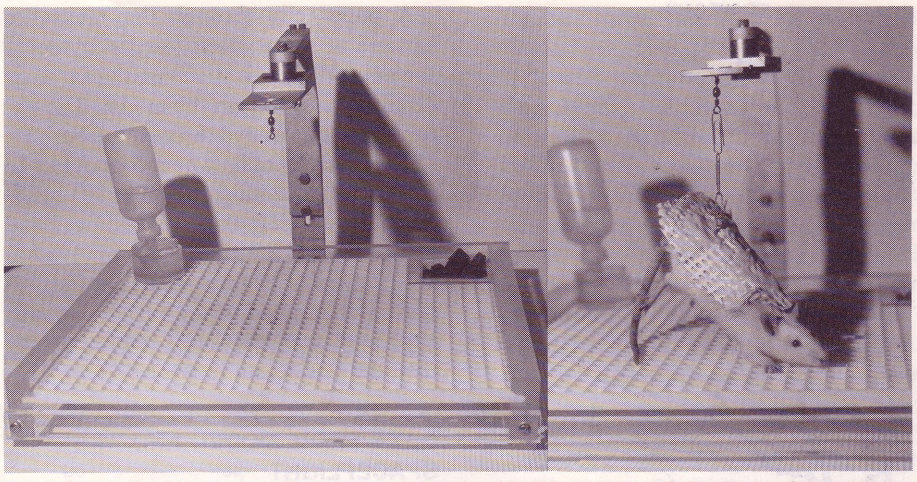

Fig. 3. Left: The present rat housing unit for the model system. Right: A rat after a 20-day suspension period.

These data suggest that the primary defect in bone turnover in young, growing rats during spaceflight occurs in calcification and that the deficiency is corrected upon return to earth. They also imply that mechanical loading is essential for normal bone turnover. However, many questions remain unanswered: What caused the decreased formation rate; was it totally due to removal of mechanical loading or did endocrine, neural, or changes in blood flow also contribute to the response? What was the total skeletal response, i.e., did it differ in nonweightbearing bones, and did total skeletal mass decrease? Did the formation rate actually cease or merely slow down? What would be the response in adult animals? Such questions could not be addressed in long-duration spaceflight experiments until the latter part of the 1980s.

RAT MODELS: A NEW APPROACH

Responses to the near-weightless environment of orbital spaceflight are virtually impossible to study under terrestrial conditions because a 1-G environment is pervading on earth. About the time proposals were submitted for Cosmos 782, animal modeling, a commonly exploited technique for investigating physiological mechanisms, was proposed for studying spaceflight-associated phenomena; data from spaceflight would be available for comparison to determine the validity of the model system. However, such a model was dependent on the development of a device emulating at least some aspects of weightlessness.

Previous attempts with the rat as a test specimen seemed unsatisfactory; total body immobilization or constraint permitted little or no health-maintaining activity. Requirements for an acceptable system included (a) the ability of the animal to exercise using *only* the front limbs (in a pulling, but nonweightbearing mode); (b) *total* unloading of the rear limbs without paralysis; (c) a fluid shift; (d) the ability to eat, drink, and groom as normally as possible; and (e) a less stressful system than those presently existing.

The system, which has evolved over the last three years, is shown in Fig. 3. This model is unique in that the animal is free to move about a 360° arc. The rat can pull himself along the plastic mesh with his front paws to reach food and regular laboratory chow; the rear limbs are totally unloaded but unrestrained. The animal is suspended in a head-down mode to perpetrate a fluid shift similar to that seen during orbital spaceflight.

The rat is attached to the model via a freely rotating fishline swivel on an overhanging horizontal aluminum beam. The beam is fixed to a cantilevered aluminum post by a ball bearing rotating in a horizontal plane. The post can be adjusted vertically to compensate for the animal's size and to keep its rear limbs off the grid.

TABLE 1. Comparison of bone parameters in Cosmos 782 and 936.

Group	782 Flight	936 Flight	936 Flight centrifuge
Endosteal bone resorption	NS*	NS	NS
% change medullary area (from control)	(+12%)	(−2%)	(+9%)
Periosteal bone formation rate			
Flight period			
% decrease from control	47%	43%	36%
Postflight period			
% increase over vivarium	53%	60%	83%
% increase over flight control	No data	NS(2%)	NS(16%)
Flight time required to form total bone volume at control rate (days)	11.7	11.1	10.7

*NS = not significantly different from control values.

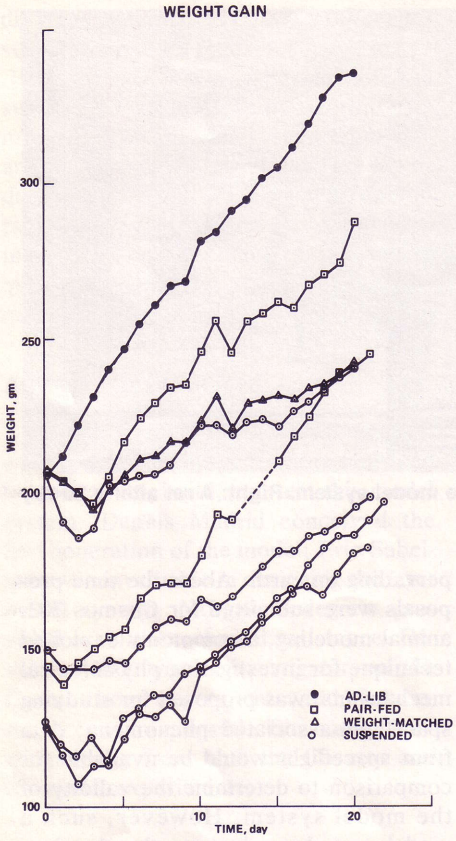

Fig. 4. Weight gain in suspended and control rats. Each data point is the average of 8–10 rats. The dashed line represents missing data points.

The water bottle can be graduated so that water consumption can be measured. A plastic plug on the bottom of the water dish allows positioning of the water bottle anywhere on the plastic grid. The plastic food dish snaps out so that food can be easily weighed. The plastic grid slides out and can be replaced or washed; the grid is sufficiently deep so that rats cannot grasp it and pull. Plastic-lined paper can be stacked underneath the model to catch urine and feces; the top paper can be removed and discarded as necessary. This model system has proven efficient, easy to use, and easy to clean.

The harness is made of a perforated, orthopedic casting material, Hexcelite, which is like plastic wrap when placed in hot water but regains its semirigid structure when cooled. The harness design can be precut, warmed, molded to the contour of the rats' shaved backs, and then bonded in place with RTV silicone rubber. Additional silicone rubber is applied to encapsulate the Hexcelite so that the animal does not catch its nails or teeth in the mesh. The bond remains functional for about two weeks. After two weeks, the harness can be removed by cutting through the new-grown hair and replaced if desired.

A paper clip through the Hexcelite harness at the posterior end provides a system for quickly attaching the rat to or disconnecting it from the model. The paper clip is positioned through the harness so that the animal, when connected to the model, maintains about a 30° head-down tilt to shift fluids, intestines, and organs toward the chest. This innovation was suggested when Soviet scientists reported that head-down tilt in humans simulated weightlessness more closely than did horizontal bedrest.

DATA FROM MODEL AND SPACEFLIGHT

Four experiments with suspension periods of approximately 20 days have been conducted to date; this time-period was chosen because it was similar to the duration of Soviet Cosmos biological satellite missions. Weight gain data from these experiments are shown in Fig. 4. Initial average weights of rats were approximately 125 g in the first two experiments, about 150 g in the third experiment, and just over 200 g in the last session. Suspended rats, regardless of their starting size, lost weight the first 2–4 days on the harness. Then they began to gain weight, and within 7–10 days all suspended groups had at least returned to their initial weight.

During the first two sessions weight-matched controls were used; these animals were found to require less food to attain the same body weight as suspended rats. During the third experiment, when harnessed and control rats received the same amount of food (pair-fed), the controls consistently gained about 20% more weight. In the fourth sessions, *ad libitum* controls gained the most weight; pair-fed controls gained about 20% more weight than suspended rats, and about 20% less food was required to weight-match control animals to the suspended animals. Thus, the weight-gain data showed that the younger the rat when harnessed, the more weight it gained while suspended, and that suspended rats gained less weight per gram of food consumed than did cage controls.

Table 2 compares weight gain and food consumption data from suspended rats with those from Cosmos rats. When these comparisons were made initially, only data from Cosmos 782 were available. At that time, it became obvious that Cosmos rats, like rats on the model (and humans in space), gained less weight per gram of food consumed than did ground controls. Pair-fed ground controls in Cosmos 782 gained about 20% more weight than did flight rats.

Similar results were predicted for Cosmos 936. However, when the data were received, the results were puzzling. Both flight rats and pair-fed ground controls appeared to have gained weight at about the same rate. Finally, word was received that the feeders had malfunctioned during flight and, unexpectedly, gave the rats more food than anticipated. Recalculation of food consumption with the revised data showed, once again, that flight rats gained less weight per gram of food consumed than did ground controls. Thus, the rat model appeared to be very useful in predicting certain metabolic costs of spaceflight.

Table 3 contains bone measurements from suspended and Cosmos rats. To measure periosteal bone formation rate, flight rats were given tetracycline (a fluorescent bone marker that binds to mineralizing bone) three days before flight because of preflight scheduling, but suspended rats were given their injection at the beginning of the experimental period. At the end of each experimental period, rats were either sacrificed or given a

TABLE 2. Cosmos and rat model: body weight and food consumption.*

Group	N†	Experimental period (days)	Initial body weight (g)	Rate of weight gain (g/day)	Rate of food consumption (g/day)
Suspended	9	20	205 ± 11.1	1.7 ± 0.8	18.3
Pair-fed	9	20	208 ± 10.8	3.6 ± 0.6	18.3
Weight-matched	10	20	208 ± 10.7	1.8 ± 0.6	14.5
Cosmos 782	11	23	225 ± 8.8	1.5 ± 0.6	14.3‡
Pair-fed	7	23	186 ± 12.0	3.8 ± 0.4	14.4‡
Cosmos 936	10	22	212 ± 5.1	3.3 ± 0.6	18.8‡
Pair-fed	10	26	194 ± 9.6	3.7 ± 0.6	16.4‡

*Data are expressed as mean ± standard deviation.
†Number of animals.
‡These rats were fed a paste diet; the food was calculated in terms of dry weight by assuming that 1 g dry weight of food = 2.7 g of paste diet.

TABLE 3. Cosmos and suspended rat model bone parameters.*

Item	Suspended	Flight	
		936	782
Number of animals	6	10	11
Initial body weight (g)	205 ± 11.1	212 ± 5.1	225 ± 8.8
Periosteal bone formation rate (R_{PBF})			
% decrease from:			
Flight control	—	43	47
Pair-fed control	44	—	—
Weight-matched control	37	—	—
Days required to form total bone volume at:			
Flight control R_{PBF}	—	11.1	11.7
Pair-fed control R_{PBF}	11.3	—	—
Weight-matched R_{PBF}	12.5	—	—
Arrest line length (mm)	2.0 ± 1.1	4.0 ± 1.1	5.3 ± 0.6
(adjusted for length of control arrest line)	(2.0)	(2.4)	(3.2)
% of periosteal surface	26	52	73
(adjusted for control)	(26)	(30)	(44)
Endosteal bone resorption (medullary area)			
% change from:			
Flight control	—	NS(−4)	NS(+12)
Pair-fed control	NS(−8)†	—	—
Weight-matched control	NS(+2)	—	—

*Data expressed as mean ± standard deviation.
†NS = not significantly different from control values.

second tetracycline label so that readaptation could be monitored. Mineralized cross sections were prepared from the tibiofibular junction. The total bone area between the tetracycline label (similar to a tree ring) and periosteal surface or between two labels was measured and divided by the number of days of the experiment to give the rate of periosteal bone formation during the experiment. Thus, periosteal formation rate is an average for the total experimental period.

Rats that weighed the most at the beginning of the experiment had the lowest bone formation rate (205 g = 36.8×10^{-3} mm³/day, 212 g = 25.5×10^{-3} mm³/day, 225 g = 15.8×10^{-3} mm³/day) suggesting that bone formation decreased as the animals matured. Suspension appeared to inhibit bone formation more dramatically in older rats. Bone formation in 125-g suspended rats decreased only about 25% from weight-matched controls, whereas formation in 200-g rats was retarded about 40%. Also, arrest lines were not detected in young (125-g) suspended rats; these lines, which indicate a cessation of formation, were first noted around the periosteum of flight rats in Cosmos 782. However, when older harnessed rats (205 g) were used, arrest lines were found in all suspended rats.

No arrest lines were found in any control group in the suspension experiments, although in the flight experiments indications of arrest lines were visible in control rats. This discrepancy may have been due to differences in age, strain of rats, and environmental factors. When comparing arrest line lengths between flight and suspended rats (Table 3), if one corrects for the arrest line in corresponding control animals, then arrest line lengths in all groups are very similar. The older rats had the most extensive arrest lines. If bone formation were to cease completely, one might expect the arrest line to extend completely around the periosteal surface. This did not occur in any experimental groups (Table 3). However, if flight values are corrected for control responses, then the percent of periosteal surface covered by an arrest line is very similar to the percent decrease in periosteal bone formation rate. If rats cease forming bone during suspension or flight, the earliest that cessation could occur would be 11 days; the total bone volume formed by the suspended or flight rats would have been accrued by the control animals in 11 days. This implies that bone formation was constant prior to arrest; however, formation probably decreased gradually rather than suddenly stopping.

DATA FROM OTHER LABORATORIES

Models have been sent to other laboratories. Data below have been obtained by X. Joseph Musacchia, professor of Physiology and Biophysics, University of Louisville, and Vojin P. Popovic, professor of Physiology, Emory University.

Musacchia and his collaborators have concentrated on renal function, water, and electrolyte balance in suspended rats (Meininger et al. 1978). For ease of urine and fecal collection, the model was modified so that the animal moved through only a 180° arc and could not turn around. In the first 2–3 days of suspension, they found (a) negative water, sodium, and potassium balance; (b) decreased water intake with no corresponding decrease in urine volume; and (c) increased urinary excretion rates of urea, ammonia, and 3-methylhistidine. Within 3–4 days, electrolyte balance became normal. Calcium balance was positive throughout the suspension period.

Their suspended rats also lost weight the first 3 days of suspension but then gained weight. Serum sodium decreased, urea increased, and potassium, calcium, and osmolality in the serum were normal at the end of 7 days of suspension. With the exception of the calcium data, most values were very similar to those reported during Skylab.

Popovic has concentrated on cardiovascular parameters using chronically cannulated rats (Popovic and Popovic 1960, Popovic et al. 1963). His preliminary data in suspended rats indicated a decrease in resting mean arterial blood pressure (which was reflected in increased right arterial pressure), absence of diurnal variation in heart rate and mean arterial blood pressure, and suggestions of increased cardiac output during the first 2–3 days on the harness. These data indicate that a fluid shift is occurring in suspended rats.

CONCLUSIONS

The most important components of the model system would appear to be unloading of the limbs and head-down tilt. These characteristics were common to all units, although harness materials and radii of movement differed between laboratories. Weight-gain data in suspended rats from all laboratories were comparable. Of the data obtained thus far, the results anticipated from Skylab data included negative water balance, fluid shift, negative potassium balance, negative nitrogen balance, muscle atrophy with increased catabolism, and increased metabolic cost. Decreased bone formation rates from the model were very similar to those of rats flown in the Cosmos biological satellites. Initial unanticipated observations from the model have included that (a) older rats responded more dramatically to suspension (and perhaps weightlessness) than young rats and (b) gut absorption of water, calcium, so-

dium, potassium, and possibly other substances was decreased.

Thus, many of the responses noted in suspended animals indicate that the model closely mimics results from rats and man exposed to near-weightlessness during orbital spaceflight. Data from experiments using the model system seemingly will allow preliminary answers to questions posed by spaceflight experiments.

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